

In the Claims:

1 (Withdrawn). A novel use of a template-dependent polymerase, the novel use comprising the step of employing said template-dependent polymerase for incorporating at least one oligonucleotide triphosphate onto a nascent oligonucleotide-3'-OH in a template-dependent manner.

2 (Withdrawn). The novel use of claim 1, wherein said at least one oligonucleotide triphosphate includes at least two mononucleotides.

3 (Withdrawn). The novel use of claim 1, wherein at least one of said at least one oligonucleotide triphosphate includes a functional group.

4 (Withdrawn). The novel use of claim 1, wherein at least one of said at least one oligonucleotide triphosphate includes at least one feature selected from the group consisting of at least one nucleotide analog having a sugar analog, at least one nucleotide analog having a base analog and at least one nucleotide analog having an internucleosidyl linkage analog.

5 (Withdrawn). The novel use of claim 1, wherein said template-dependent polymerase is selected from the group consisting of DNA-dependent DNA polymerase, DNA-dependent RNA polymerase, RNA-dependent DNA polymerase and RNA-dependent RNA polymerase.

6 (Withdrawn). The novel use of claim 5, wherein said template-dependent polymerase is thermostable.

7 (Withdrawn). A method of identifying a template-dependent polymerase having increased activity in incorporating oligonucleotide triphosphates onto a nascent oligonucleotide-3'-OH in a template-dependent manner, among a library of mutated template-dependent polymerases, the method comprising the step of screening the library using template-dependent polymerization of oligonucleotide triphosphates for

selecting a template-dependent polymerase mutant exhibiting increased activity in incorporating the oligonucleotide triphosphates onto the nascent oligonucleotide-3'-OH in a template-dependent manner.

8 (Withdrawn). The method of claim 7, further comprising the steps of using said template-dependent polymerase mutant as a basis for creating a second library of mutated template-dependent polymerases derived therefrom and screening the second library using said template-dependent polymerization of oligonucleotide triphosphates for selecting a second template-dependent polymerase mutant demonstrating yet increased activity in incorporating the oligonucleotide triphosphates onto the nascent oligonucleotide-3'-OH in a template-dependent manner.

9 (Withdrawn). The method of claim 8, wherein said library of mutated template-dependent polymerases is created using at least one method selected from the group of random mutagenesis, random fragments shuffling and gene-family shuffling of genes corresponding to protein fragments and/or domains.

10 (Withdrawn). A method of assaying a template-dependent polymerase for its activity in incorporating oligonucleotide triphosphates onto a nascent oligonucleotide-3'-OH in a template-dependent manner, the method comprising the step of using template-dependent polymerization of oligonucleotide triphosphates for assaying the template-dependent polymerase for its activity in incorporating oligonucleotide triphosphates onto the nascent oligonucleotide-3'-OH in the template-dependent manner.

11 (Withdrawn). A method of better exploiting the information transfer capacity of a nucleic acid molecule, the method comprising the step of synthesizing a complementary nucleic acid molecule employing oligonucleotide triphosphates instead of, or in addition to, nucleotide triphosphates, as basic units for synthesis.

12 (Original). A method of extending a nascent oligonucleotide-3'-OH in a template-dependent manner, the method comprising the step of contacting the nascent

oligonucleotide-3'-OH with a nucleic acid template, a template-dependent polymerase and at least one oligonucleotide triphosphate under conditions in which said nascent oligonucleotide-3'-OH hybridizes with said nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate onto a growing 3'-OH group of the nascent oligonucleotide-3'-OH, thereby extending the nascent oligonucleotide-3'-OH in a template-dependent manner.

13 (Withdrawn). A method of amplifying a nucleic acid template, the method comprising the step of contacting the nucleic acid template with a nascent oligonucleotide-3'-OH, a template-dependent polymerase and at least one oligonucleotide triphosphate under conditions in which said nascent oligonucleotide-3'-OH hybridizes with the nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate onto a growing 3'-OH group of said nascent oligonucleotide-3'-OH, thereby amplifying the nucleic acid template.

14 (Withdrawn). A method of detecting a sequence alteration in a nucleic acid template, the method comprising the step of contacting a nascent oligonucleotide-3'-OH with the nucleic acid template, a template-dependent polymerase and at least one oligonucleotide triphosphate under conditions in which said nascent oligonucleotide-3'-OH hybridizes with said nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate onto a growing 3'-OH group of the nascent oligonucleotide-3'-OH, thereby extending the nascent oligonucleotide-3'-OH in a template-dependent manner, said at least one oligonucleotide triphosphate being selected so as to enable extending the nascent oligonucleotide-3'-OH in the template-dependent manner only if the sequence alteration is present, or in the alternative, only if the sequence alteration is absent.

15 (Withdrawn). A method of detecting the presence or absence of a sequence alteration in a nucleic acid template, the method comprising the steps of:

- (a) contacting the nucleic acid template with a nascent oligonucleotide-3'-OH, a template-dependent polymerase and at least one oligonucleotide

triphosphate under conditions in which said nascent oligonucleotide-3'-OH hybridizes with the nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate onto a growing 3'-OH group of said nascent oligonucleotide-3'-OH if appropriate base pairing exists between the nucleic acid template and said oligonucleotide triphosphate, and said template-dependent polymerase is substantially inactive in incorporating said at least one oligonucleotide triphosphate onto said growing 3'-OH group of said nascent oligonucleotide-3'-OH if appropriate base pairing fails to exist between the nucleic acid template and said at least one oligonucleotide triphosphate; and

- (b) detecting whether said at least one oligonucleotide triphosphate is incorporated onto said growing 3'-OH group of said nascent oligonucleotide-3'-OH thereby detecting the presence or absence of the sequence alteration in the nucleic acid template.

16 (Original). A method of extending a nascent oligonucleotide-3'-OH in a template-dependent manner, the method comprising the step of contacting the nascent oligonucleotide-3'-OH with a nucleic acid template, a template-dependent polymerase and 4^N oligonucleotide triphosphates each including N monomers, wherein N is an integer greater than 1, under conditions in which said nascent oligonucleotide-3'-OH hybridizes with said nucleic acid template and said template-dependent polymerase is active in incorporating said oligonucleotide triphosphates onto a growing 3'-OH group of the nascent oligonucleotide-3'-OH, thereby extending the nascent oligonucleotide-3'-OH in a template-dependent manner.

17 (Withdrawn). A method of amplifying a nucleic acid template, the method comprising the step of contacting the nucleic acid template with a nascent oligonucleotide-3'-OH, a template-dependent polymerase and 4^N oligonucleotide triphosphates each including N monomers, wherein N is an integer greater than 1, under conditions in which said nascent oligonucleotide-3'-OH hybridizes with the nucleic acid template and said template-dependent polymerase is active in

incorporating said at least one oligonucleotide triphosphate onto a growing 3'-OH group of said nascent oligonucleotide-3'-OH, thereby amplifying the nucleic acid template.

18 (Withdrawn). A method of exponentially amplifying a nucleic acid template, the method comprising the step of contacting the nucleic acid template with a pair of nascent oligonucleotides-3'-OH, said nascent oligonucleotides-3'-OH being hybridizable with opposite strands of the nucleic acid template, a template-dependent polymerase and 4^N oligonucleotide triphosphates each including N monomers, wherein N is an integer greater than 1, under conditions in which said nascent oligonucleotides-3'-OH hybridize with said opposite strands of the nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate onto a growing 3'-OH group of each of said nascent oligonucleotides-3'-OH, thereby exponentially amplifying the nucleic acid template.

19 (Original). A method of extending a nascent oligonucleotide-3'-OH in a template-dependent manner, the method comprising the step of contacting the nascent oligonucleotide-3'-OH with a nucleic acid template, a template-dependent polymerase, at least one oligonucleotide triphosphate and at least one nucleotide triphosphate, wherein said at least one oligonucleotide triphosphate and said at least one nucleotide triphosphate are selected such that at least one monomer of said at least one oligonucleotide triphosphate is absent from said at least one nucleotide triphosphate, under conditions in which said nascent oligonucleotide-3'-OH hybridizes with said nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate and said at least one nucleotide triphosphate onto a growing 3'-OH of the nascent oligonucleotide-3'-OH, thereby extending the nascent oligonucleotide-3'-OH in a template-dependent manner.

20 (Withdrawn). A method of amplifying a nucleic acid template, the method comprising the step of contacting the nucleic acid template with a nascent oligonucleotide-3'-OH, a template-dependent polymerase, at least one oligonucleotide

triphosphate and at least one nucleotide triphosphate, wherein said at least one oligonucleotide triphosphate and said at least one nucleotide triphosphate are selected such that at least one monomer of said at least one oligonucleotide triphosphate is absent from said at least one nucleotide triphosphate, under conditions in which said nascent oligonucleotide-3'-OH hybridizes with the nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate onto a growing 3'-OH group of said nascent oligonucleotide-3'-OH, thereby amplifying the nucleic acid template.

21 (Withdrawn). A method of detecting a sequence alteration in a nucleic acid template, the method comprising the step of contacting the nascent oligonucleotide-3'-OH with a nucleic acid template, a template-dependent polymerase, at least one oligonucleotide triphosphate and at least one nucleotide triphosphate, wherein said at least one oligonucleotide triphosphate and said at least one nucleotide triphosphate are selected such that at least one monomer of said at least one oligonucleotide triphosphate is absent from said at least one nucleotide triphosphate, under conditions in which said nascent oligonucleotide-3'-OH hybridizes with said nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate onto the 3'-OH group of the nascent oligonucleotide-3'-OH, thereby extending the nascent oligonucleotide-3'-OH in a template-dependent manner, said at least one oligonucleotide triphosphate being selected so as to enable extending the nascent oligonucleotide-3'-OH in a template-dependent manner only if the sequence alteration is present, or in the alternative, only if the sequence alteration is absent.

22 (Withdrawn). A method of detecting the presence or absence of a sequence alteration in a nucleic acid template, the method comprising the steps of:

- (a) contacting the nucleic acid template with a nascent oligonucleotide-3'-OH, a template-dependent polymerase, at least one oligonucleotide triphosphate and at least one nucleotide triphosphate, wherein said at least one oligonucleotide triphosphate and said at least one nucleotide triphosphate are selected such that at least one monomer of said at least

one oligonucleotide triphosphate is absent from said at least one nucleotide triphosphate, under conditions in which said nascent oligonucleotide-3'-OH hybridizes with the nucleic acid template and said template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate and said at least one nucleotide triphosphate onto a growing 3'-OH group of said nascent oligonucleotide-3'-OH if appropriate base pairing exists between the nucleic acid template and said at least one oligonucleotide triphosphate, and said template-dependent polymerase is substantially inactive in incorporating said at least one oligonucleotide triphosphate onto said growing 3'-OH group of said nascent oligonucleotide-3'-OH if appropriate base pairing fails to exist between the nucleic acid template and said at least one oligonucleotide triphosphate; and

- (b) detecting whether said oligonucleotide triphosphate is incorporated onto said growing 3'-OH group of said nascent oligonucleotide-3'-OH, thereby detecting the presence or absence of the sequence alteration in the nucleic acid template.

23 (Withdrawn). A method of determining a sequence of a nucleic acid template, the method comprising the steps of:

- (a) contacting in one or more reaction vessels the nucleic acid template with a nascent oligonucleotide-3'-OH, a template-dependent polymerase, 4^N oligonucleotide triphosphates each including N monomers, 4^N oligonucleotide triphosphate analogs each including N monomers of which a 3' monomer includes a chain terminator moiety, wherein N is an integer greater than 1, under conditions in which said nascent oligonucleotide-3'-OH hybridizes with the nucleic acid template and said template-dependent polymerase is active in incorporating said oligonucleotide triphosphates and said oligonucleotide triphosphate analogs onto a growing 3'-OH group of said nascent oligonucleotide-3'-OH, so as to obtain a population of nucleic acid chains each being terminated by a different oligonucleotide

triphosphate analog of said 4^N oligonucleotide triphosphate analogs;
and

- (b) size separating said population of terminated nucleic acid chains, thereby determining the sequence of the nucleic acid template.

24 (Withdrawn). The method of claim 23, wherein each of said 4^N oligonucleotide triphosphate analogs is labeled by a unique label.

25 (Withdrawn). The method of claim 24, wherein at least some of said unique labels is combinatorial labels.

26 (Withdrawn). A method of engineering functional nucleic acid polymers and polypeptides by introduction of multiple point mutations in nucleic acid sequences, as a novel approach of directed evolution, the method comprising the steps of:

- (a) contacting in reaction vessels a nucleic acid template with a nascent oligonucleotide-3'-OH, a template-dependent polymerase, and 4^N oligonucleotide triphosphates, each including N monomers, wherein N is an integer greater than 1, and wherein at least one of said oligonucleotide triphosphates has a mismatch as compared to the template sequence, under conditions in which the nascent oligonucleotide-3'-OH hybridizes with the nucleic acid template and the template-dependent polymerase is active in incorporating said oligonucleotide triphosphates and said at least one oligonucleotide triphosphate containing said mismatch onto a growing 3'-OH group of the nascent oligonucleotide-3'-OH, so as to obtain a population of nucleic acid chains each containing one or multiple mutations; and
- (b) amplifying said mutated population of nucleic acid chains and further shuffling, cloning and expressing them by methods known in the art to create pools of degenerate nucleic acid sequences and of degenerate polypeptides; and
- (c) screening said pools for individual clones with desired properties, and

then using the selected clones as precursors for additional cycles of degeneration and selection, as described above, until the selected molecules are optimized for the desired function.

27 (Withdrawn). A method of better exploiting the information transfer and functional capacities of nucleic acid molecules for DNA chip technology and nanotechnology, the method comprising the step of contacting a component selected from at least one nucleic acid template, at least one template-dependent polymerase, at least one nascent oligonucleotide-3'-OH, at least one oligonucleotide triphosphate and/or at least one oligonucleotide triphosphate analog, wherein at least one of said components is attached onto a solid support used in a nanodevice or DNA chip, and wherein said at least one template-dependent polymerase is active in incorporating said at least one oligonucleotide triphosphate and/or said at least one oligonucleotide triphosphate analog onto said growing 3'-OH group of said nascent oligonucleotide-3'-OH, so as to obtain a population of nucleic acid chains bound to the solid support, which can be further manipulated by means as described above including, but not limited to, template-dependent extension, template-dependent amplification, detection of sequence alteration, and detection of nucleic acid sequences.

28 (Withdrawn). A composition comprising 4^N oligonucleotide triphosphates each having N monomers, wherein N is an integer greater than 1.

29 (Withdrawn). The composition of claim 28, wherein each of said 4^N oligonucleotide triphosphates includes at least two monomers.

30 (Withdrawn). The composition of claim 28, wherein at least one of said 4^N oligonucleotide triphosphates includes a functional group.

31 (Withdrawn). The composition of claim 28, wherein at least one of said 4^N oligonucleotide triphosphates includes at least one feature selected from the group consisting of at least one nucleotide analog having a sugar analog, at least one nucleotide analog having a base analog and at least one nucleotide analog having an

internucleosidyl linkage analog.

32 (Withdrawn). A composition comprising at least one oligonucleotide triphosphate and at least one nucleotide triphosphate, wherein said at least one oligonucleotide triphosphate and said at least one nucleotide triphosphate are selected such that monomers forming said least one oligonucleotide triphosphate are not represented among said at least one nucleotide triphosphate and *vice versa*.

33 (New). The method of claim 16, wherein N equals 2 or 3.